SEQUENCE LISTING

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<120> Microbial production of L-ascorbic acid

<130> 21864 WO

<150> EP 03017677.0
<151> 2003-08-14

<160> 31

<170> PatentIn version 3.2

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<213> Gluconobacter oxydans N44-1

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Leu Gly Gly Ser Trp Phe Tyr Thr Leu Ala Gly Ile Ala Leu Ala Ala
35 40 45

Ser Ser Val Tyr Met Ile Arg Arg Asn Ile Leu Ser Thr Trp Ile Ala
50 55 60

Leu Gly Leu Leu Val Ala Thr Ala Leu Trp Ser Leu Ala Glu Val Gly

65 70 75 80

Thr Ser Phe Trp Pro Ser Phe Ser Arg Leu Ile Val Phe Leu Cys Val
85 90 95

Ala Leu Ile Ala Thr Leu Met Ala Pro Trp Leu Ser Gly Pro Gly Arg
100 105 110

Arg Tyr Phe Thr Arg Pro Val Thr Gly Ala Thr Ser Gly Ala Leu Gly
115 120 125

Ala Ile Ile Val Ala Phe Leu Ala Gly Met Phe Arg Val His Pro Thr
130 135 140

Ile Ala Pro Gln Asp Thr Thr His Pro Gln Glu Thr Ala Ser Thr Ala
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Asp Ser Asp Gln Pro Gly His Asp Trp Pro Ala Tyr Gly Arg Thr Ala
165 170 175

Ser Gly Thr Arg Tyr Ala Ser Phe Thr Gln Ile Asn Arg Asp Asn Val
180 185 190

Ser Lys Leu Arg Val Ala Trp Thr Tyr Arg Thr Gly Asp Met Ala Leu
195 200 205

Asn Gly Ala Glu Phe Gln Gly Thr Pro Ile Lys Ile Gly Asp Thr Val
210 215 220

Tyr Ile Cys Ser Pro His Asn Ile Val Ser Ala Leu Asp Pro Asp Thr
225 230 235 240

Gly Thr Glu Lys Trp Lys Phe Asp Pro His Ala Gln Thr Lys Val Trp
245 250 255

Gln Arg Cys Arg Gly Val Gly Tyr Trp His Asp Ser Thr Ala Thr Asp
260 265 270

Ala Asn Ala Pro Cys Ala Ser Arg Ile Val Leu Thr Thr Ile Asp Ala
275 280 285

Arg Leu Ile Thr Ile Asp Ala Arg Thr Gly Gln Ala Cys Thr Asp Phe
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Gly Thr Asn Gly Asn Val Asn Leu Leu Thr Gly Leu Gly Pro Thr Ala
305 310 315 320

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Val Val Val Gly Gly Arg Ile Ala Asp Asn Glu Arg Thr Gly Glu Pro
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Ser Gly Val Val Arg Gly Tyr Asp Val Arg Thr Gly Ala Gln Val Trp
        355                 360                 365

Ala Trp Asp Ala Thr Asn Pro His Arg Gly Thr Thr Pro Leu Ala Glu
    370                 375                 380

Gly Glu Ile Tyr Pro Ala Glu Thr Pro Asn Met Trp Gly Thr Ala Ser
385                 390                 395                 400

Tyr Asp Pro Lys Leu Asn Leu Val Phe Phe Pro Leu Gly Asn Gln Thr
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Pro Asp Phe Trp Gly Gly Asp Arg Ser Lys Ala Ser Asp Glu Tyr Asn
            420                 425                 430

Asp Ala Phe Val Ala Val Asp Ala Lys Thr Gly Asp Glu Arg Trp His
        435                 440                 445

Phe Arg Thr Ala Asn His Asp Leu Val Asp Tyr Asp Ala Thr Ala Gln
    450                 455                 460

Pro Ile Leu Tyr Asp Ile Pro Asp Gly His Gly Gly Thr Arg Pro Ala
465                 470                 475                 480

Ile Ile Ala Met Thr Lys Arg Gly Gln Ile Phe Val Leu Asp Arg Arg
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Asp Gly Thr Pro Ile Val Pro Val Glu Met Arg Lys Val Pro Gln Asp
            500                 505                 510

Gly Ala Pro Glu His Gln Tyr Leu Ala Pro Glu Gln Pro Tyr Ser Ala
        515                 520                 525

Leu Ser Ile Gly Thr Glu Arg Leu Lys Pro Ser Asp Met Trp Gly Gly
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Thr Ile Phe Asp Gln Leu Leu Cys Arg Ile Gln Phe Ala Ser Tyr Arg
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Tyr Glu Gly Glu Phe Thr Pro Val Asn Glu Lys Gln Ala Thr Ile Ile
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Tyr Pro Gly Tyr Tyr Gly Gly Ile Asn Trp Gly Gly Gly Ala Val Asp
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Gly Lys Phe Met Lys Gln Glu Glu Ala Arg Arg Ser Gly Phe Lys Pro
    610                 615                 620

Ser Ser Glu Gly Glu Tyr Ser Glu Gln Lys Gly Thr Pro Trp Gly Val
625                 630                 635                 640

Val Arg Ser Met Phe Phe Ser Pro Ala Gly Leu Pro Cys Val Lys Pro
                645                 650                 655

Pro Tyr Gly Thr Met Asn Ala Ile Asp Leu Arg Ser Gly Lys Val Lys
            660                 665                 670

Trp Ser Met Pro Leu Gly Thr Ile Gln Asp Met Pro Val His Gly Met
        675                 680                 685

Val Pro Gly Leu Ala Ile Pro Leu Gly Met Pro Thr Met Ser Gly Pro
    690                 695                 700

Leu Ala Thr His Thr Gly Leu Val Phe Phe Ser Gly Thr Leu Asp Asn
705                 710                 715                 720

Tyr Val Arg Ala Leu Asn Thr Asp Thr Gly Glu Val Val Trp Lys Ala
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Arg Leu Pro Val Ala Ser Gln Ala Ala Pro Met Ser Tyr Met Ser Asp
            740                 745                 750

Lys Thr Gly Lys Gln Tyr Ile Val Val Thr Ala Gly Gly Leu Thr Arg
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acccagcgat atgtggggcg gcacgatctt cgaccagctc ctgtgccgca tccagttcgc 240

ctcctaccgc tatgaaggcg agttcacccc cgtcaacgag aagcaggcca ccatcatcta 300

tccgggctat tacggcggca tcaactgggg cggcggcgcc gtggatgaaa gcaccggaac 360

gctgctggtc aacgacatcc gcatggccca gtggggcaag ttcatgaagc aagaagaagc 420

ccgccgcagc ggcttcaaac ccagctcgga aggcgaatat tccgaacaga aaggcacccc 480

ctggggcgtc gtccgctcga tgttcttctc ccccgccggt ctcccctgcg tgaaaccgcc 540

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aatgccgacc atgagcggcc cgctggccac ccataccggc ctggtcttct tctccggcac 720

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Pro Gln Asp Gly Ala Pro Glu His Gln Tyr Leu Ala Pro Glu Gln Pro
        35                  40                  45

Tyr Ser Ala Leu Ser Ile Gly Thr Glu Arg Leu Lys Pro Ser Asp Met
    50                  55                  60

Trp Gly Gly Thr Ile Phe Asp Gln Leu Leu Cys Arg Ile Gln Phe Ala
65                  70                  75                  80

Ser Tyr Arg Tyr Glu Gly Glu Phe Thr Pro Val Asn Glu Lys Gln Ala
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Thr Ile Ile Tyr Pro Gly Tyr Tyr Gly Gly Ile Asn Trp Gly Gly Gly
            100                 105                 110

Ala Val Asp Glu Ser Thr Gly Thr Leu Leu Val Asn Asp Ile Arg Met
        115                 120                 125

Ala Gln Trp Gly Lys Phe Met Lys Gln Glu Glu Ala Arg Arg Ser Gly
    130                 135                 140

Phe Lys Pro Ser Ser Glu Gly Glu Tyr Ser Glu Gln Lys Gly Thr Pro
145                 150                 155                 160

Trp Gly Val Val Arg Ser Met Phe Phe Ser Pro Ala Gly Leu Pro Cys
                165                 170                 175

Val Lys Pro Pro Tyr Gly Thr Met Asn Ala Ile Asp Leu Arg Ser Gly
            180                 185                 190

Lys Val Lys Trp Ser Met Pro Leu Gly Thr Ile Gln Asp Met Pro Val
        195                 200                 205

His Gly Met Val Pro Gly Leu Ala Ile Pro Leu Gly Met Pro Thr Met
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Ser Gly Pro Leu Ala Thr His Thr Gly Leu Val Phe Phe Ser Gly Thr
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gtntacatga tccgtcgcaa catcctctcg acatggatcg ccctcggcct gcttgtggca 180

acagccctgt ggtcgctcgc cgaagtcggc accagcttct ggcccagctt ctcccgcctg 240

atcgtatttc tgtgcgtcgc cctgatcgcg accctcatgg cgccctggct cagcggcccc 300

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Gly Ile Ala Leu Ala Ala Ser Ser Val Tyr Met Ile Arg Arg Asn Ile
35 40 45

Leu Ser Thr Trp Ile Ala Leu Gly Leu Leu Val Ala Thr Ala Leu Trp
50 55 60

Ser Leu Ala Glu Val Gly Thr Ser Phe Trp Pro Ser Phe Ser Arg Leu
65 70 75 80

Ile Val Phe Leu Cys Val Ala Leu Ile Ala Thr Leu Met Ala Pro Trp
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Leu Ser Gly Pro Gly Arg Arg Tyr Phe Thr Arg Pro Val Thr Gly Ala
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Thr Ser Gly Ala
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tctcggccct cgaccccgat accggcacgg aaaagtggaa gttcgacccc cacgcccaga 180

cgaaagtctg gcagcgctgc cgcggcgtcg gctactggca tgacagcacg gccacggacg 240

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Ile Cys Ser Pro His Asn Ile Val Ser Ala Leu Asp Pro Asp Thr Gly
35 40 45

Thr Glu Lys Trp Lys Phe Asp Pro His Ala Gln Thr Lys Val Trp Gln
50 55 60

Arg Cys Arg Gly Val Gly Tyr Trp His Asp Ser Thr Ala Thr Asp Ala
65 70 75 80

Asn Ala Pro Cys Ala Ser Arg Ile Val Leu Thr Thr Ile Asp Ala Arg
85 90 95

Leu Ile Thr Ile Asp Ala Arg Thr Gly Gln Ala Cys Thr Asp Phe Gly
100 105 110

Thr Asn Gly Asn Val Asn Leu Leu Thr Gly Leu Gly Pro Thr Ala Pro
115 120 125

Gly Ser Tyr Tyr Pro Thr Ala Ala Pro Leu Val Ala Gly Asp Ile Val
130 135 140

Val Val Gly Gly Arg Ile Ala Asp Asn Glu Arg Thr Gly Glu Pro Ser
145 150 155 160

Gly Val Val Arg Gly Tyr Asp Val Arg Thr Gly Ala Gln Val Trp Ala
165 170 175

Trp Asp Ala Thr Asn Pro His Arg Gly Thr Thr Pro Leu Ala Glu Gly
180 185 190

Glu Ile Tyr Pro Ala Glu Thr Pro Asn Met Trp Gly Thr Ala Ser Tyr
195 200 205

Asp Pro Lys Leu Asn Leu Val Phe Phe Pro Leu Gly Asn Gln Thr Pro
210 215 220

Asp Phe Trp Gly Gly Asp Arg Ser Lys Ala Ser Asp Glu Tyr Asn Asp
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Glu Gln Pro Tyr Ser Ala Leu Ser Ile Gly Thr Glu Arg Leu Lys Pro
35 40 45

Ser Asp Met Trp Gly Gly Thr Ile Phe Asp Gln Leu Leu Cys Arg Ile
50 55 60

Gln Phe Ala Ser Tyr Arg Tyr Glu Gly Glu Phe Thr Pro Val Asn Glu
65 70 75 80

Lys Gln Ala Thr Ile Ile Tyr Pro Gly Tyr Tyr Gly Gly Ile Asn Trp
85 90 95

Gly Gly Gly Ala Val Asp Glu Ser Thr Gly Thr Leu Leu Val Asn Asp
100 105 110

Ile Arg Met Ala Gln Trp Gly Lys Phe Met Lys Gln Glu Glu Ala Arg
115 120 125

Arg Ser Gly Phe Lys Pro Ser Ser Glu Gly Glu Tyr Ser Glu Gln Lys
130 135 140

Gly Thr Pro Trp Gly Val Val Arg Ser Met Phe Phe Ser Pro Ala Gly
145 150 155 160

Leu Pro Cys Val Lys Pro Pro Tyr Gly Thr Met Asn Ala Ile Asp Leu
165 170 175

Arg Ser Gly Lys Val Lys Trp Ser Met Pro Leu Gly Thr Ile Gln Asp
180 185 190

Met Pro Val His Gly Met Val Pro Gly Leu Ala Ile Pro Leu Gly Met
195 200 205

Pro Thr Met Ser Gly Pro Leu Ala Thr His Thr Gly Leu Val Phe Phe
210 215 220

Ser Gly Thr Leu Asp Asn Tyr Val Arg Ala Leu Asn Thr Asp Thr Gly
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Glu Val Val Trp Lys Ala Arg Leu Pro Val Ala Ser Gln Ala Ala Pro
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gtntacatga tccgtcgcaa catcctctcg acatggatcg ccctcggcct gcttgtagca 180

acagccctgt ggtcgctcgc cgaagtcggc accagcttct ggcccagctt ctcccgcctg 240

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Gly Ile Ala Leu Ala Ala Ser Ser Val Tyr Met Ile Arg Arg Asn Ile
35 40 45

Leu Ser Thr Trp Ile Ala Leu Gly Leu Leu Val Ala Thr Ala Leu Trp
50 55 60

Ser Leu Ala Glu Val Gly Thr Ser Phe Trp Pro Ser Phe Ser Arg Leu
65 70 75 80

Ile Val Phe Leu Cys Val Ala Leu Ile Ala Thr Leu Met Ala Pro Trp
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Thr Ser Gly Ala Leu Gly Ala Ile
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<400> 22

Arg Asp Asn Val Ser Lys Leu Arg Val Ala Trp Thr Tyr Arg Thr Gly
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Asp Met Ala Leu Asn Gly Ala Glu Phe Gln Gly Thr Pro Ile Lys Ile
20 25 30

Gly Asp Thr Val Tyr Ile Cys Ser Pro His Asn Ile Val Ser Ala Leu
35 40 45

Asp Pro Asp Thr Gly Thr Glu Lys Trp Lys Phe Asp Pro His Ala Gln
50 55 60

Thr Lys Val Trp Gln Arg Cys Arg Gly Val Gly Tyr Trp His Asp Ser
65 70 75 80

Thr Ala Thr Asp Ala Asn Ala Pro Cys Ala Ser Arg Ile Val Leu Thr
85 90 95

Thr Ile Asp Ala Arg Leu Ile Thr Ile Asp Ala Arg Thr Gly Gln Ala
100 105 110

Cys Thr Asp Phe Gly Thr Asn Gly Asn Val Asn Leu Leu Thr Gly Leu
115 120 125

Gly Pro Thr Ala Pro Gly Ser Tyr Tyr Pro Thr Ala Ala Pro Leu Val
130 135 140

Ala Gly Asp Ile Val Val Val Gly Gly Arg Ile Ala Asp Asn Glu Arg
145 150 155 160

Thr Gly Glu Pro Ser Gly Val Val Arg Gly Tyr Asp Val Arg Thr Gly
165 170 175

Ala Gln Val Trp Ala Trp Asp Ala Thr Asn Pro His Arg Gly Thr Thr
180 185 190

Pro Leu Ala Glu Gly Glu Ile Tyr Pro Ala Glu Thr Pro Asn Met Trp
195 200 205

Gly Thr Ala Ser Tyr Asp Pro Lys Leu Asn Leu Val Phe Phe Pro Leu
210 215 220

Gly Asn Gln Thr Pro Asp Phe Trp Gly Gly Asp Arg Ser Lys Ala Ser
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Asp Glu Tyr Asn Asp Ala Phe Val Ala Val Asp Ala
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<213> Artificial

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<213> Artificial

<220>
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<210> 25
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<213> Artificial

<220>
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<400> 25
gcacgctcga caactatgtc 20

<210> 26
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<213> Gluconobacter oxydans IFO 3244

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ctcgccggca tcgcgctggc ggccagcagc gtctacatga tccgtcgcaa catcctctcg 180

acatggatcg ccctcggcct gcttgtagca acagccctgt ggtcgctcgc cgaagtcggc 240
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atgagcggcc cgctggccac ccataccggc ctggtcttct tctccggcac gctcgacaac 2160

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gcctcacagg ccgctccgat gagctacatg tccgacaaga ccggcaaaca gtacatcgtc 2280

gtcaccgcag gcggcctgac ccgctccggc gtcgacaaaa accgcggcga ctacgtcatc 2340

gcctacgccc tgccctccga agaataa 2367

<210> 27
<211> 788
<212> PRT
<213> Gluconobacter oxydans IFO 3244

<400> 27

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Ala Leu Met Ala Ala Phe Leu Ile Ile Glu Gly Leu His Leu Ile Ile
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Leu Gly Gly Ser Trp Phe Tyr Thr Leu Ala Gly Ile Ala Leu Ala Ala
35 40 45

Ser Ser Val Tyr Met Ile Arg Arg Asn Ile Leu Ser Thr Trp Ile Ala
50 55 60

Leu Gly Leu Leu Val Ala Thr Ala Leu Trp Ser Leu Ala Glu Val Gly
65 70 75 80

Thr Ser Phe Trp Pro Ser Phe Ser Arg Leu Ile Val Phe Leu Cys Val
85 90 95

Ala Leu Ile Ala Thr Leu Met Ala Pro Trp Leu Ser Gly Pro Gly Arg
100 105 110

Arg Tyr Phe Thr Arg Pro Val Thr Gly Ala Thr Ser Gly Ala Leu Gly
115 120 125

Ala Ile Ile Val Ala Phe Leu Ala Gly Met Phe Arg Val His Pro Thr
130 135 140

Ile Ala Pro Gln Asp Thr Thr His Pro Gln Glu Thr Ala Ser Thr Ala
145 150 155 160

Asp Ser Asp Gln Pro Gly His Asp Trp Pro Ala Tyr Gly Arg Thr Ala
165 170 175

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Ser Gly Thr Arg Tyr Ala Ser Phe Thr Gln Ile Asn Arg Asp Asn Val
            180                 185                 190

Ser Lys Leu Arg Val Ala Trp Thr Tyr Arg Thr Gly Asp Met Ala Leu
        195                 200                 205

Asn Gly Ala Glu Phe Gln Gly Thr Pro Ile Lys Ile Gly Asp Thr Val
    210                 215                 220

Tyr Ile Cys Ser Pro His Asn Ile Val Ser Ala Leu Asp Pro Asp Thr
225                 230                 235                 240

Gly Thr Glu Lys Trp Lys Phe Asp Pro His Ala Gln Thr Lys Val Trp
                245                 250                 255

Gln Arg Cys Arg Gly Val Gly Tyr Trp His Asp Ser Thr Ala Thr Asp
            260                 265                 270

Ala Asn Ala Pro Cys Ala Ser Arg Ile Val Leu Thr Thr Ile Asp Ala
        275                 280                 285

Arg Leu Ile Thr Ile Asp Ala Arg Thr Gly Gln Ala Cys Thr Asp Phe
    290                 295                 300

Gly Thr Asn Gly Asn Val Asn Leu Leu Thr Gly Leu Gly Pro Thr Ala
305                 310                 315                 320

Pro Gly Ser Tyr Tyr Pro Thr Ala Ala Pro Leu Val Ala Gly Asp Ile
                325                 330                 335

Val Val Val Gly Gly Arg Ile Ala Asp Asn Glu Arg Thr Gly Glu Pro
            340                 345                 350

Ser Gly Val Val Arg Gly Tyr Asp Val Arg Thr Gly Ala Gln Val Trp
        355                 360                 365

Ala Trp Asp Ala Thr Asn Pro His Arg Gly Thr Thr Pro Leu Ala Glu
    370                 375                 380

Gly Glu Ile Tyr Pro Ala Glu Thr Pro Asn Met Trp Gly Thr Ala Ser
385                 390                 395                 400

Tyr Asp Pro Lys Leu Asn Leu Val Phe Phe Pro Leu Gly Asn Gln Thr
                405                 410                 415

Pro Asp Phe Trp Gly Gly Asp Arg Ser Lys Ala Ser Asp Glu Tyr Asn
            420                 425                 430
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Asp Ala Phe Val Ala Val Asp Ala Lys Thr Gly Asp Glu Arg Trp His
      435                 440                 445

Phe Arg Thr Ala Asn His Asp Leu Val Asp Tyr Asp Ala Thr Ala Gln
    450                 455                 460

Pro Ile Leu Tyr Asp Ile Pro Asp Gly His Gly Gly Thr Arg Pro Ala
465                 470                 475                 480

Ile Ile Ala Met Thr Lys Arg Gly Gln Ile Phe Val Leu Asp Arg Arg
                485                 490                 495

Asp Gly Thr Pro Ile Val Pro Val Glu Met Arg Lys Val Pro Gln Asp
            500                 505                 510

Gly Ala Pro Glu His Gln Tyr Leu Ala Pro Glu Gln Pro Tyr Ser Ala
        515                 520                 525

Leu Ser Ile Gly Thr Glu Arg Leu Lys Pro Ser Asp Met Trp Gly Gly
    530                 535                 540

Thr Ile Phe Asp Gln Leu Leu Cys Arg Ile Gln Phe Ala Ser Tyr Arg
545                 550                 555                 560

Tyr Glu Gly Glu Phe Thr Pro Val Asn Glu Lys Gln Ala Thr Ile Ile
                565                 570                 575

Tyr Pro Gly Tyr Tyr Gly Gly Ile Asn Trp Gly Gly Gly Ala Val Asp
            580                 585                 590

Glu Ser Thr Gly Thr Leu Leu Val Asn Asp Ile Arg Met Ala Gln Trp
        595                 600                 605

Gly Lys Phe Met Lys Gln Glu Glu Ala Arg Arg Ser Gly Phe Lys Pro
    610                 615                 620

Ser Ser Glu Gly Glu Tyr Ser Glu Gln Lys Gly Thr Pro Trp Gly Val
625                 630                 635                 640

Val Arg Ser Met Phe Phe Ser Pro Ala Gly Leu Pro Cys Val Lys Pro
                645                 650                 655

Pro Tyr Gly Thr Met Asn Ala Ile Asp Leu Arg Ser Gly Lys Val Lys
            660                 665                 670

Trp Ser Met Pro Leu Gly Thr Ile Gln Asp Met Pro Val His Gly Met
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675 680 685

Val Pro Gly Leu Ala Ile Pro Leu Gly Met Pro Thr Met Ser Gly Pro
690 695 700

Leu Ala Thr His Thr Gly Leu Val Phe Phe Ser Gly Thr Leu Asp Asn
705 710 715 720

Tyr Val Arg Ala Leu Asn Thr Asp Thr Gly Glu Val Val Trp Lys Ala
725 730 735

Arg Leu Pro Val Ala Ser Gln Ala Ala Pro Met Ser Tyr Met Ser Asp
740 745 750

Lys Thr Gly Lys Gln Tyr Ile Val Val Thr Ala Gly Gly Leu Thr Arg
755 760 765

Ser Gly Val Asp Lys Asn Arg Gly Asp Tyr Val Ile Ala Tyr Ala Leu
770 775 780

Pro Ser Glu Glu
785

<210> 28
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<220>
<223> Primer

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<210> 29
<211> 30
<212> DNA
<213> Artificial

<220>
<223> Primer

<400> 29
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<210> 30
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<212> DNA
<213> Artificial

<220>
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<400> 30
aagtcatatg aacagcggcc cccgcacgct 30

<210> 31
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<220>
<223> Primer

<400> 31
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